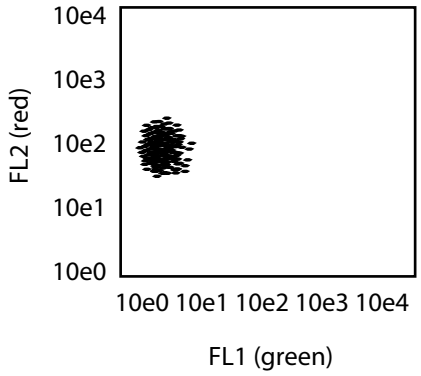
A Short Introduction to Compensation

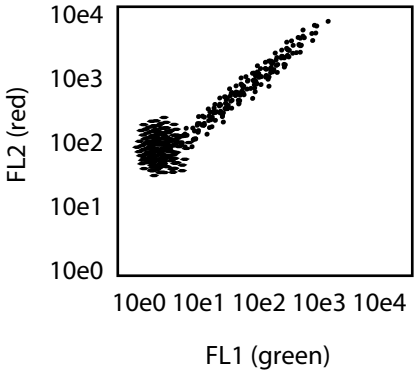
Let's say you are detecting an endogenous cell surface marker with a PE-conjugated antibody. If you measure fluorescence using FL1 and FL2, you will see something like this:

Fig. 1



If you also transfect the cells with GFP and perform the same labeling, you will see something like this:

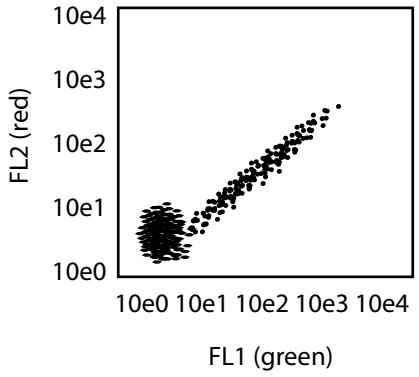
Fig. 2



What has happened is that the green channel (FL1) has bled into the red channel due to spectral overlap. The more GFP (green) the cells express, the more the signal leaks over into the FL2 channel. This is fixed by COMPENSATION.

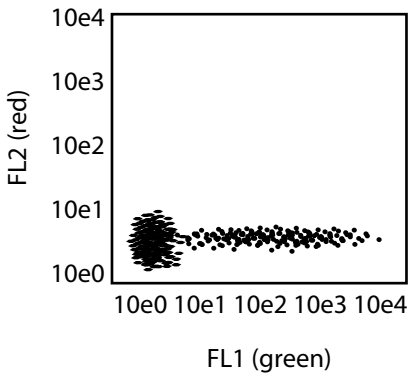
The simplest way to do this is to take GFP-transfected cells that are NOT labeled with antibody. They should look like this:

Fig. 3



Open the Compensation window and adjust the FL2 - FL1% parameter until the cells look like this:

Fig. 4



In effect, what you are doing is reducing the measured FL2 parameter in each cell by a percentage of the FL1 parameter for that cell, eliminating the contribution of spectral overlap. Note that there is also an FL1 - FL2% parameter in the Compensation window. This is harder to adjust. Using 1.0% for this parameter is a good default.

Tricks to get good compensation:

If you increase the voltage on the FL1 PMT you will reduce the amount of compensation you need.

Conversely, if you increase the voltage on the FL2 PMT, you will need more compensation.

Judicious use of FL2 - FL1%, FL1 voltage, and FL2 voltage will get you good compensation.

Good luck!

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